

09/214868

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TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER 47114

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)

INTERNATIONAL APPLICATION NO.
PCT/EP 97/03571

INTERNATIONAL FILING DATE
7 July 1997

PRIORITY DATE CLAIMED
18 July 1996

TITLE OF INVENTION: SOLID SUPPORTS FOR ANALYTICAL MEASURING PROCESSES, A PROCESS FOR THEIR
PREPARATION, AND THEIR USE

APPLICANT(S) FOR DO/EO/US Heinz EISEL, Harald KELLER

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. /X/ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
 2. / / This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
 3. /X/ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
 4. /X / A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
 5. /X/ A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
 - a./X/ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b./ / has been transmitted by the International Bureau.
 - c./ / is not required, as the application was filed in the United States Receiving Office (RO/USO).
 6. /X/ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
 7. /X/ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
 - a./X/ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b./ / have been transmitted by the International Bureau.
 - c./ / have not been made; however, the time limit for making such amendments has NOT expired.
 - d./ / have not been made and will not be made.
 8. /X/ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
 9. /X/ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
 10. / / A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).
- Items 11. to 16. below concern other document(s) or information included:
- 11./X/ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
 - 12./X/ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
 - 13./X/ A FIRST preliminary amendment.
/ / A SECOND or SUBSEQUENT preliminary amendment.
 - 14./ / A substitute specification.
 - 15./ / A change of power of attorney and/or address letter.
 - 16./X/ Other items or information.
International Search Report
International Preliminary Examination Report

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U.S. Appln. No. (If Known) INTERNATIONAL APPLN. NO.
PCT/EP 97/03571

ATTORNEY'S DOCKET NO.
47114

		<u>CALCULATIONS</u>	<u>PTO USE ONLY</u>
17. /X/ The following fees are submitted			
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):			
Search Report has been prepared by the EPO or JPO.....		\$840.00	840.00
International preliminary examination fee paid to USPTO (37 CFR 1.482).....		\$750.00	
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).....		\$700.00	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO		\$970.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....		\$98.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =		\$	840.00
Surcharge of \$130.00 for furnishing the oath or declaration later than / / 20 / / 30 months from the earliest claimed priority date (37 CFR 1.492(e)).			
<u>Claims</u>	<u>Number Filed</u>	<u>Number Extra</u>	<u>Rate</u>
Total Claims	8 -20		X\$18.
	1		
Indep.Claims	-3		X\$78.
Multiple dependent claim(s) (if applicable)		+260.	
TOTAL OF ABOVE CALCULATION		=	840.00
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).			
SUBTOTAL		=	840.00
Processing fee of \$130. for furnishing the English translation later than / / 20 / / 30 months from the earliest claimed priority date (37 CFR 1.492(f)).			
TOTAL NATIONAL FEE		=	840.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) \$40.00 per property			
TOTAL FEES ENCLOSED		= \$	880.00
		Amount to be	
		refunded:	\$
		Charged	\$

a./X/ A check in the amount of \$ 880.00 to cover the above fees is enclosed.

b./ / Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c./X/ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 11-0345. A duplicate copy of this sheet is enclosed.

+

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
KEIL & WEINKAUF
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SIGNATURE

Herbert B. Keil
NAME

18,967
Registration No.

09/214868

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of
EIPEL et al.

BOX PCT

International Application
PCT/EP 97/03571

Filed: July 7, 1997

For: SOLID SUPPORTS FOR ANALYTICAL MEASURING PROCESSES, A PROCESS
FOR THEIR PREPARATION, AND THEIR USE

PRELIMINARY AMENDMENT

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

Prior to examination, kindly amend the above-identified
application as follows.

IN THE CLAIMS

Page 11 of the claims, indicated line 35, replace "11.
[sic]" with --7.--;

indicated line 39, replace "12.[sic]"
with --8--.

Claim 3, line 1, delete "or 2" and insert --claim 1--.

Claim 4, line 1, delete "any of claims 1 to 3" and insert
--claim 1--.

Claim 5, line 1, delete "any of claims 1 to 4" and insert
--claim 1--.

Newly renumbered claim 7, lines 1 and 2, delete "or 6".

Newly renumber claim 8, line 1, delete "any of claims 1 to
4" and insert --claim 1--.

R E M A R K S

Claims 1-12 were amended in the international preliminary
examination. An English language translation of the amended
claims is attached. The claims were incorrectly numbered in the

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amendment, and the instant preliminary amendment corrects the misnumbering. The claims have been further amended to eliminate multiple dependency and to put them in better form for U.S. filing. No new matter is included.

Favorable action is solicited.

Respectfully submitted,

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Solid supports for analytical measurement methods, their production and their use

- 5 The invention relates to solid supports for analytical measurement methods which are essentially composed of an inert solid support material on which hydrophilic measurement zones which may be provided with a surface loading are separated from one another by at least one hydrophobic coating, where the number of measurement points applied per cm^2 of the support is greater than or equal to 10. The invention furthermore relates to a process for producing the supports, and to the use of the supports in diagnostic methods, in research looking for active substances, in combinatorial chemistry, in crop protection, in toxicology or in environmental protection.

A main task of research looking for active substances in crop protection or in medicine is to identify novel lead structures and to develop active substances derived from these structures.

- 20 In classical research looking for active substances, the biological effect of novel compounds has been tested in random screening on the whole organism, for example the plant or the microorganism. Employed for this purpose were complex in vitro and in vivo test methods with which only a few hundred substances could be tested each year.

In this case the biological testing was the limiting factor with respect to the synthetic chemistry.

- 30 The provision of molecular test systems by molecular and cell biology has led to a drastic change in the situation. These molecular test systems, such as receptor binding assays, enzyme assays or cell-cell interaction assays, can, as a rule, readily be carried out in microtiter plates in reaction volumes of from 50 to 250 μl and can easily be automated. Automation and miniaturization of these test systems permits the sample throughput to be high. This development makes it possible to test large numbers of different chemicals for possible use as lead structure in research looking for active substances.

- A modern automated test system allows 100,000 or more chemicals to be tested for their biological effect each year in mass screening. Microtiter plate assays are very often used because, as a rule, they entail low costs, are very reliable and have little susceptibility to faults.

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In order to be able fully to exploit the efficiency of these test systems, novel solid-phase syntheses have been and are still being developed in combinatorial chemistry.

- 5 Combinatorial chemistry makes it possible to synthesize a wide variety of different chemical compounds, called substance libraries. This is particularly true when combinatorial chemistry makes use of automated solid-phase synthesis (see, for example, review articles I. [sic] Med. Chem. 37 (1994) 1233 and 1385).
- 10 Solid-phase synthesis has the advantage that a large number of compounds can be synthesized, and that by-products and excess reactants can easily be removed, so that elaborate purification of the products is unnecessary.
- 15 The large number of synthesized compounds in combinatorial chemistry means that the efficiency of modern automated test systems can be fully exploited with regard to chemical diversity. Since, however, in contrast to classical active substance synthesis, the chemicals to be investigated are not available in any desired
- 20 amount on synthesis by means of combinatorial chemistry, only a restricted number of test systems can be examined because of the amounts of chemicals required in the test systems.

- Another disadvantage of present test systems, for example in re-
- 25 search looking for active substances, in diagnostic methods, in environmental protection or crop protection, is that the reagents required for many test systems, such as enzymes, antibodies, receptors, fluorescent dyes, radioactively or otherwise labeled ligands, cytokines, activators, inhibitors or other reagents, are
- 30 costly, difficult to prepare and/or not available in a quantity sufficient for the automated tests.

DE-A 44 35 727 describes an approach for reducing the reagents required for a test.

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The disadvantage of this process is that the support for the measurements must first be produced in an elaborate multistage process.

- 40 Another disadvantage is that the reactions which can be carried out with this support material are confined to reactions linked to solid phases, such as reactant binding between antibodies, antigens, haptens or nucleic acids. It is not possible with this method to carry out reactions in solution.

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It is an object of the present invention to develop a novel analytical measurement method which can be carried out without the stated disadvantages and provide it for research looking for active substances, diagnostic methods, environmental protection, crop protection, toxicology or combinatorial chemistry.

We have found that this object is achieved by using the solid support described at the outset for the measurement method.

- 10 It has been found that the surface tension which hinders further miniaturization of the present microtiter plate technique to ever smaller reaction cavities (= wells), because thereby forces such as adhesion of the reaction liquid to the surface of the microtiter plates or the capillary forces are of increasing importance, and thus make it impossible to fill the reaction cavities and thus carry out a measurement, in very small microtiter plate wells, can be utilized advantageously for the supports according to the invention.
- 20 Hydrophilic measurement zones on the support mean areas on the support on which or in which the measurement is carried out after application of the reaction liquid and thus of the reactants (see number 2 in Figures 1, 3 and 4). They thus correspond to the wells in microtiter plates and are referred to hereinafter as
- 25 "measurement zones or measurement points".

- The hydrophilic measurement zones on the support are advantageously surrounded by a hydrophobic zone (see number 1 in Figures 1 to 4). This hydrophobic zone can be composed of at least one
- 30 hydrophobic coating which covers the support completely or only partly with discontinuities. These discontinuities (see number 5 in Figures 1 to 4) are advantageously hydrophilic.

Figures 1 to 4 serve to illustrate the supports according to the invention by way of example.

- The measurement zones, and the hydrophobic zones which separate them from one another (see number 1 in Figures 1 to 4), can be applied, for example, by microlithography, photoetching, microprinting or a micropunch technique or can be sprayed on using a mask technique. Photochemical processes which can be used to make the surfaces of plates or rolls specifically hydrophobic at particular points and hydrophilic at other points are known from the techniques for producing printing plates. It is possible with
- 40 this technique to produce, for example, a grid of several thousand regularly arranged hydrophilic measurement zones (see number 2 in Figures 1, 3 and 4), surrounded by hydrophobic margins (see

number 1 in Figures 1 to 4), in a simple manner on a support, eg. on a glass or metal plate. This may entail firstly one or more hydrophobic coatings being applied to the support, and subsequently the measurement zones being applied to the required
5 points or, conversely, initially the hydrophilic measurement zones and then the hydrophobic zones, or both simultaneously, being applied. It is also possible to apply a plurality of hydrophilic measurement zones to the same point.

- 10 Figure 2 depicts by way of example a support according to the invention having the size of a microtiter plate.

The measurement zones can have any desired shape, with circular measurement zones being preferred.

15

The hydrophobic coating or coatings may be applied coherently to the support or else be provided with discontinuities of any design. They may also be in the form of separate zones around the measurement zones, with hydrophobic rings separating the hydro-

- 20 philic measurement zones from one another being preferred.

The hydrophobic coating or coatings are intended to prevent the measurement zones spreading into one another and thus to make accurate measurement of individual reaction mixtures possible.

25

It is possible in principle to apply any desired number of measurement points onto a support, but the number of measurement points per cm² is preferably greater than or equal to 10, particularly preferably greater than or equal to 15 and very particularly preferably greater than or equal to 20. Moreover, the reaction volumes applied are from a few nl up to some µl, with volumes of less than 5 µl being preferred, and of less than or equal to 1 µl being particularly preferred.

- 30
35 The measurement points can be applied in any desired grids to the support, and square or rectangular grids are preferred.

The inert solid support may consist of a level, planar plate of a block of the same type or of a sheet of any desired shape and

- 40 size, which may have small depressions (see Figure 4) at the measurement zone points, with flat supports (see Figure 3) being preferred. Rectangular or square supports are preferred, and rectangular supports with the size of a standard microtiter plate (127.5 mm x 85.5 mm) or integral multiples of microtiter plates,
45 which can be larger or smaller, for example the Terasaki plates (81 mm x 56 mm, 60 measurement points), are particularly preferred. The preferred size of the supports according to the in-

vention has the advantage that all the peripherals of automated microtiter plate technology can be used without alteration.

The support may consist, for example, of materials such as glass,
5 ceramic, quartz, metal, stone, wood, plastic, rubber, silicon,
germanium or porcelain. The materials can be used in pure form,
as mixtures, alloys or blends or in various layers or after coat-
ing with, for example, a plastic or a paint for producing the
supports according to the invention. Transparent supports made of
10 quartz, glass, plastic, germanium or silicon, which are suitable
for all visual tests such as microscopic, camera-assisted and
laser-assisted tests, are preferably produced.

Suitable transparent plastics are all amorphous plastic materials
15 which [lacuna] in a single-phase or multiphase manner with iden-
tical refractive index as polymers of acrylonitrile/butadiene/
styrene or in a multiphase manner with different refractive in-
dex, in which the domains of the plastic components form zones
which are smaller than the wavelength of light, such as the block
20 copolymers of polystyrene and butadiene (polystyrene/butadiene
blends).

Particularly suitable transparent plastics which may be mentioned
in this connection are polystyrene, styrene/acrylonitrile, poly-
25 propylene, polycarbonate, PVC (= polyvinyl chloride), poly(methyl
methacrylate), polyesters, silicones, polyethylene/acrylate,
polylactide or cellulose acetate, cellulose propionate, cellulose
butyrate or mixtures thereof. Silicon or germanium supports are
particularly suitable for applications in which detection or in-
30 duction of the reaction using near infrared light is necessary.

The support according to the invention may also be designed in
the form of a conveyor belt which, when the assays are automated,
can move past the charging, incubation or detection stations.

35 One process for producing the supports according to the invention
starts, for example, from ceramic, quartz or glass plates. The
support is for this purpose expediently first cleaned with a
cleaner, for example an alcohol, an alkaline cleaner or an acidic
40 cleaner such as Reacalc® (which contains, according to the sup-
plier Chemotec GmbH, phosphoric acid and surfactants). The clean-
ing can advantageously be improved by carrying it out in an
ultrasonic bath. After cleaning, the support is dried, immedi-
ately or after rinsing with water and/or alcohol or with an alco-
45 hol/water mixture. The hydrophobic coating of the support takes
place, for example, with a 1 % strength hexadecyltrimethoxysilane
solution in a solvent such as isopropanol/H₂O (9:1) using a punch

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technique. The punch is briefly pressed on the glass slide to apply the 1 % strength hexadecyltrimethoxysilane solution. The support is subsequently dried. The glass support is advantageously dried at elevated temperatures, ie. above 80°C. The support is
5 preferably rinsed once again after drying to remove excess hexadecyltrimethoxysilane, for example with an alcohol/water mixture such as isopropanol/H₂O (9:1).

This punch technique may be used to apply an additional surface
10 loading in the region of the hydrophilic measurement points. This surface loading can be generated, for example, by applying proteins, acidic or basic polymers such as polylysine or acidic or basic molecules.

15 Methods suitable for applying sample material and reagents are all those able to meter amounts of liquid from a few nl to a few µl, such as techniques used in ink jet printers (see DE-A 40 24 544) or in flow cytometry, in cell sorters (Horan, P.K., Wheelless, L.L., Quantitative Single Analysis and Sorting, Science
20 198 (1977) 149-157). Drop formation can in this case take place by piezoelectric drop formation (ultrasound), piezoelectric drop ejection or ejection by evaporation (ink jet technique). It is possible to use systems with permanent drop production or systems which produce drops on demand.

25 These techniques can be used to place individual droplets in an accurately metered and targeted manner on the individual hydrophilic measurement points of the multianalysis surface of the support by, for example, moving the support under one or more
30 nozzles, which are arranged in parallel, in accordance with the rhythm of the metered liquid and in accordance with the preset grid. It is also possible likewise to move the metering device, for example consisting of at least one nozzle, over the support in accordance with the rhythm of the metered liquid and in accordance with the preset grid.
35

It is possible with these techniques if necessary to place different reagents and/or individual cells on the predetermined sites (measurement points) on the support surface and bring about
40 reaction thereof. It is advantageous that, with the small volumes preferred according to the invention, in the range from a few nanoliters to a few microliters, mixing of the reactants by diffusion takes place very quickly so that no special mechanical mixing device is necessary. It is also possible, before the addition of liquid droplets for carrying out the actual analysis, for
45 certain ligands, eg. proteins or nucleic acids, to be present on

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the support in adsorbed or chemically bound form before metering in the measurement samples and the reagents.

Further advantages of the supports according to the invention are
5 the saving of substances such as chemicals to be tested, enzymes, cells or other reactants, of time through a further increase in parallel reaction mixtures, which are automated where appropriate, of space and staff requirements, due to further miniaturization of the reaction mixtures and thus finally also of money.

10

The droplets placed on the supports may also be applied in the form of gel droplets which subsequently solidify where appropriate and thus reduce evaporation of the reaction liquid.

15 Evaporation of the reaction liquid (see number 3 in Figures 3 and 4) can also be reduced by coating with a hydrophobic liquid (see number 4 in Figures 3 and 4), in which case the hydrophobic coating or coatings act like an anchor (Figure 3 and 4).

Low-viscosity oils such as silicone oils are preferably used for

20 the coating.

Evaporation can also be reduced by incubating the supports in an atmosphere which is virtually saturated with water vapor.

25 Reduction in evaporation is likewise possible by cooling the supports.

Evaporation can be reduced by using single elements of those mentioned or combinations thereof.

30

The supports according to the invention are suitable in principle for all analytical methods now carried out in microtiter plates, such as colorimetric, fluorimetric or densitometric methods. It is possible in these cases to use and measure light scattering,
35 turbidity, wavelength-dependent light absorption, fluorescence, luminescence, raman scattering, ATR (= Attenuated Total Reflection), radioactivity, isotope labeling, pH shifts or ion shifts, advantageously alone or in combination, to mention only a few of the possible measured quantities here.

40

Analytical methods which can be carried out on the supports according to the invention and which may be mentioned here are the binding of antibodies to antigens, the interaction between receptors and ligands, the specific cleavage of substrate molecules by
45 enzymes, the polymerase chain reaction (PCR), agglutination tests or the interaction between different or identical cell types such as enzyme assays, titration assays such as virus titration

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5 The supports according to the invention can be employed, for example, in diagnostic methods, in research looking for active substances, in combinatorial chemistry, in crop protection, in toxicology, in environmental protection, for example for cytotoxicological tests, in medicine or in biochemistry.

The supports according to the invention are particularly suitable for mass screening.

15

20 Example 1

25 Firstly, the glass slide was cleaned with a 20 % strength aqueous solution of an acidic cleaner (Reacalc® supplied by Chemotec GmbH) in an ultrasonic immersion bath for 10 minutes. The glass slide was subsequently rinsed with water and then with absolute ethanol and dried at about 23°C.

A micropunch was used to apply the hydrophobic coating in the form of hydrophobic rings (see Figure 1 to 4) on the hydrophilic support. The hydrophobic layer was applied using a 1 % strength hexadecyltrimethoxysilane solution in isopropanol/H₂O (9:1). The punch was dipped in the silane solution and then briefly, for about 5 sec, pressed on the support, and then the support was dried at 100°C for 15 minutes. Excess silane solution was removed from the support by immersing the support in isopropanol/H₂O (9:1) for about 1 minute. Two types of punches were used to apply 12 and 25, respectively, measurement points per square centimeter.

45

Example 2

Protease inhibitor assay with the supports according to the invention

A protease inhibitor assay was carried out using a support produced by the process described in Example 1.

- 96 samples each comprising 100 nl of a solution of casein labeled with fluorescein isothiocyanate (20 µg/ml) in 10 mM tris/HCl buffer (pH 8.5) were applied, in a chamber with a relative humidity exceeding 95 % using a micrometering system supplied by Microdrop, Norderstedt, to a slide produced as described above. The reaction droplets were arranged in 8 rows and 12 columns in a 2 x 2 mm grid in accordance with the hydrophobic zones (= barrier layers) applied by the punch. The width of the hydrophobic rings was 0.4 mm in each case.
- Subsequently, 1 nl of each of various protease inhibitors in a concentration of 1 mM in 10 mM tris/HCl buffer (pH 8.5) was added to the reaction samples using the Microdrop apparatus. Addition took place precisely into the fluorescent-labeled casein solution which had previously been applied. One nanoliter of 10 mM tris/HCl buffer (pH 8.5) was used as control.

Finally, 10 nl of the protease trypsin in a concentration of 10 mg/ml in tris/HCl buffer (pH 8.5) were added to the reaction.

- The reaction droplets were subsequently covered either with mineral oil, silicone oil or liquid paraffin, which was applied using the microdrop metering system, to reduce evaporation.

- After incubation for 30 minutes, the assay was measured. This was done using an inverted fluorescence microscope for excitation, from the underside of the slide, with linearly polarized light in the range from 450 to 485 nm and for detection of fluorescence in the range from 515 to 530 nm. A coolable CCD camera in front of which there was a polarization filter which could be rotated by a motor was used for detection.

The anisotropy of polarization of the casein molecules was determined using the following equations:

10

$$A = \frac{I_{\text{perpendicular}} - I_{\text{parallel}}}{I_{\text{perpendicular}} + 2 \times I_{\text{parallel}}} \quad (\text{I})$$

$$A = \frac{I_{\text{perpendicular}} - I_{\text{parallel}}}{I_{\text{perpendicular}} + I_{\text{parallel}}} \quad (\text{II})$$

where:

10

- A is the anisotropy
 P is the polarization
 I_{parallel} is the measured intensity of the fluorescent light on polarization parallel to the polarization of the exciting light and
 $I_{\text{perpendicular}}$ is the measured intensity of the fluorescent light with crossed polarization filters.

15

The anisotropy is a measure of the rotational diffusion coefficient of molecules and can be employed to estimate the hydrodynamic sizes of molecules (G. Weber, Biochemie, Vol. 51, 1952, 145-155).

20

On cleavage of the protein labeled with fluorescein isothiocyanate by the protease, a polarization in the range from 50 to 75×10^{-3} was measured. On inhibition of the protease trypsin, the measured polarization was greater than 150×10^{-3} .

25

For parallel analysis of all 96 reaction mixtures, the complete measurement field with the 96 points was covered at one time with a lens of a stereo magnifier and was analyzed using an image-processing program.

30

Example 3

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Protease inhibitor assay with 1536 parallel measurement points

A trypsin inhibitor assay was carried out as described in Example 2 with 1536 parallel measurement points on the size of a microtiter plate (see Figure 2).

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We claim:

1. A solid support for analytical measurement methods which is essentially composed of an inert solid support material on which hydrophilic measurement zones which may be provided with a surface loading are separated from one another by at least one hydrophobic coating, where the number of measurement points applied per cm^2 of the support is greater than or equal to 10.
2. A solid support as claimed in claim 1, wherein the hydrophilic measurement zones are separated from one another by at least one continuous hydrophobic coating.
3. A solid support as claimed in claim 1, wherein the hydrophilic measurement zones applied to the support are separated from one another by non-continuous hydrophobic zones.
4. A support as claimed in any of claims 1 to 3, wherein the support material used is glass, ceramic, quartz, metal, stone, plastic, rubber, silicon or porcelain.
5. A support as claimed in any of claims 1 to 4, wherein a transparent support material selected from the group of glass, quartz, silicon or plastic is used.
6. A process for producing a support as claimed in any of claims 1 to 5, which comprises providing the support with at least one hydrophobic coating, and applying the hydrophilic measurement zones by microlithography, photoetching, microprinting or a micropunch technique.
7. A process for producing a support as claimed in any of claims 1 to 5, which comprises providing a hydrophilic or hydrophilized support with at least one hydrophobic coating by microlithography, photoetching, microprinting or a micropunch technique so as to produce hydrophilic measurement zones which are separated from one another.
8. A process for producing a support as claimed in claim 6 or 7, wherein a surface loading is additionally applied in the hydrophilic measurement zones on the support.
9. An analytical measurement method which comprises liquid analysis samples which may, where appropriate, be covered with a hydrophobic layer being applied and analyzed in the

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hydrophilic measurement zones on a support as claimed in any of claims 1 to 5.

10. An analytical measurement method as claimed in claim 9,
5 wherein the analytical measurement is carried out in an atmosphere which is virtually saturated with water vapor.
11. An analytical measurement method as claimed in claim 9 or 10,
10 wherein the analytical measurement is carried out while cooling the support.
12. The use of a support as claimed in any of claims 1 to 5 in
15 diagnostic methods, in research looking for active substances, in combinatorial chemistry, in crop protection, in toxicology or in environmental protection.

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Solid supports for analytical measurement methods, their production and their use

5 Abstract

The invention relates to solid supports for analytical measurement methods which are essentially composed of an inert solid support material on which hydrophilic measurement zones, separated from one another by a hydrophobic coating, where at least 10 measurement points are applied to the support per cm². The invention furthermore relates to a process for producing the supports, and to the use of the supports in diagnostic methods, in research looking for active substances, in combinatorial chemistry, in crop protection, in toxicology or in environmental protection.

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FIG.1

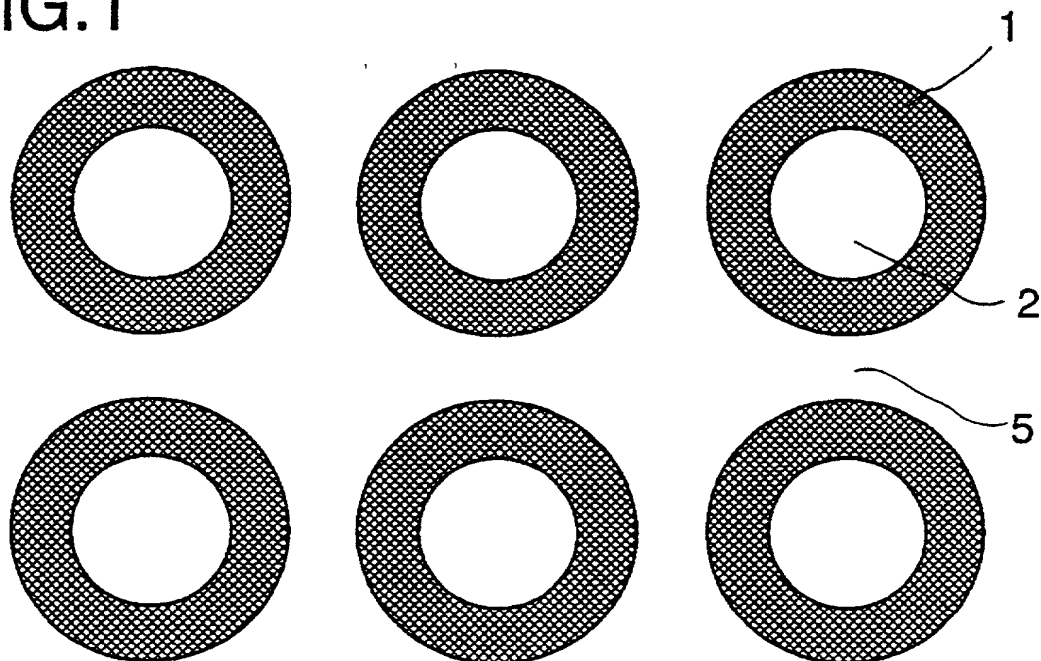


FIG.2

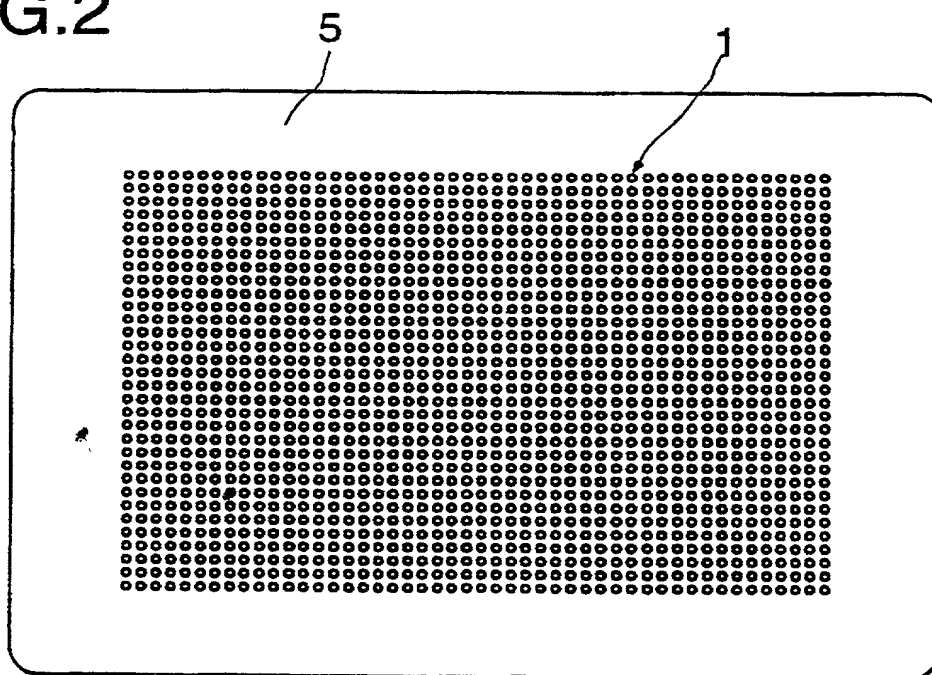


FIG.3

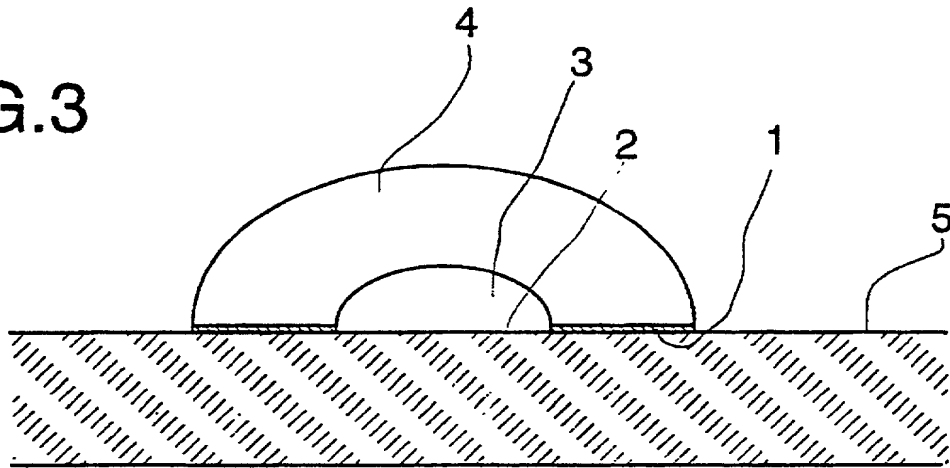
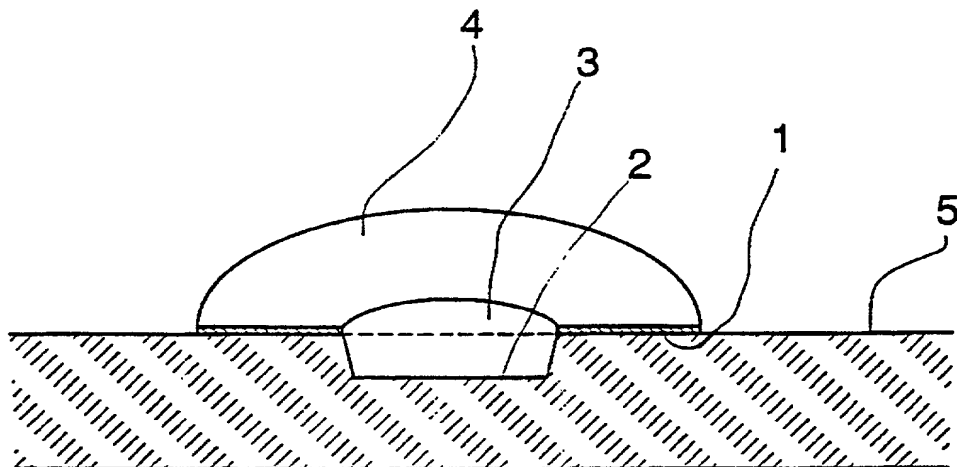


FIG.4



Declaration, Power of Attorney

Page 1 of 3

O. Z. 0050/47114

We (I), the undersigned inventor(s), hereby declare(s) that:

My residence, post office address and citizenship are as stated below next to my name,

We (I) believe that we are (I am) the original, first, and joint (sole) inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Solid supports for analytical measurement methods, their production and their use

the specification of which

☒ is attached hereto.

☐ was filed on _____ as

Application Serial No. _____

and amended on _____.

☒ was filed as PCT international application

Number _____ PCT/EP 97/03571

on _____ 07/07/1997

and was amended under PCT Article 19

on _____ (if applicable).

We (I) hereby state that we (I) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We (I) acknowledge the duty to disclose information known to be material to the patentability of this application as defined in Section 1.56 of Title 37 Code of Federal Regulations.

We (I) hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed. Prior Foreign Application(s)

Application No.	Country	Day/Month/Year	Priority Claimed
19628928.9	Federal Republic of Germany	18th July 1996	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

O. Z.0050/47114

We (I) hereby claim the benefit under Title 35, United States Codes, § 119(e) of any United States provisional application(s) listed below.

(Application Number)_____
(Filing Date)_____
(Application Number)_____
(Filing Date)

We (I) hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application Serial No.	Filing Date	Status (pending, patented, abandoned)
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

2 — And we (I) hereby appoint **Messrs. HERBERT. B. KEIL**, Registration Number 18,967; and **RUSSEL E. WEINKAUF**, Registration Number 18,495; the address of both being Messrs. Keil & Weinkauf, 1101 Connecticut Ave., N.W., Washington, D.C. 20036 (telephone 202-659-0100), our attorneys, with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to sign the drawings, to receive the patent, and to transact all business in the Patent Office connected therewith.

We (I) declare that all statements made herein of our (my) own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Heinz Eipel

Heinz Lipal
Signature of Inventor

Date 21/07/1997

Federal Republic of Germany *AEX*

Post Office Address: *same as residence*

Harald Keller

Harold Ridge

Date 21/07/1997

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